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US Army Edgewood Arsenal
Chemical Research and Development Laboratories

Technical Report

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# *Artificial-Respiration Studies in Monkeys Incapacitated by Experimental Botulism*

by

Fred W. Oberst
Paul Cresthull
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## FOREWORD

The work described in this report was authorized under Project 1C622401A097, Medical Defense Aspects of Chemical Agents (U). The experimental data are contained in notebooks MN-1687, MN-1703, and MN-1722. This work was started in January 1963 and completed in August 1964.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.


### Acknowledgments

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The services of Capt B. A. Herrero, Capt Charles Kuhn, Dr. C. S. Streett, Capt A. E. Ecklund, Lt J. Dagle, and Dr. Duane F. Ford of the Pathology Branch, who performed the autopsies on the monkeys, are acknowledged.

## DIGEST

The effect of artificial respiration (AR), with and without antitoxin, was studied in monkeys with respiratory paralysis after intravenous administrations of 5, 12, and 24 LD50's of Type A botulinum toxin. The AR was started after severe toxic signs had developed. The average times to death in both groups were markedly delayed when compared with those of the untreated control group. The animals treated with AR only gradually became less responsive, appeared to be in a coma most of the time during the last day, and eventually died. In the group receiving a combination of AR, antitoxin, and adequate nursing care, two animals developed spontaneous respiration. It was necessary to give them AR intermittently. Eventually, they died from lung consolidation or plugging of airway passages. Those animals that failed to develop spontaneous respiration died during AR from oxygen insufficiency caused by congestion or consolidation of the lungs, or both.

In monkeys with respiratory paralysis from lethal doses of botulinum toxin, AR, with and without antitoxin, aids in prolonging life; however, continued long-term treatment may result in irreversible lung damage, probably caused by the method of AR.

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## I. INTRODUCTION.

Death from botulism is caused by respiratory paralysis. Artificial respiration (AR) may be of some value when respiration becomes difficult or is about to cease. In mild cases of botulism, a tracheal cannula may improve respiratory ventilation; however, when the respiratory muscles become quite weak, mechanical assistance in respiration has been shown to be beneficial. The Drinker-type respirator (iron lung) has been used on some patients with botulism, but it has not always saved the victim.

In the present study, AR, with and without antitoxin, was performed on monkeys after intravenous (iv) injection of botulinum toxin to determine whether it is possible to reverse respiratory paralysis and to determine the complications that may be encountered with this type of therapy. Special nursing care and supportive treatment, such as intragastric (ig) and iv or intraperitoneal (ip) feedings, were standard procedures used for the majority of animals.

## II. EXPERIMENTATION.

### A. Materials.

1. Animals. A total of 19 sooty mangabey monkeys, weighing 6 to 11 kg, was used.

2. Toxin. The botulinum toxin, Type A, was similar to that described by Lamanna, McElroy, and Eklund.[1] The mouse ip LD50 (MU) for this partially purified toxin was approximately $3 \times 10^{-4}$ µg. The stock solution was prepared from the powdered material dissolved in a sterile gelatin-phosphate buffer solution (10 gm of $Na_2HPO_4$ and 2 gm of Difco gelatin in 1 ℓ of distilled water); the pH was adjusted to 6.8 by the addition of concentrated HCl. All stock solutions were assayed in mice for potency before they were released for these studies. The potency of the diluted solutions ranged from 500 to 2,000 MU/ml.

3. Antitoxin. Bivalent botulinum antitoxin (equine origin, Lederle), globulin-modified, Types A and B, 500 units/ml of each, was used.

4. Respirator (Drinker-Type). The equipment consisted of three distinct units: respirator cylinder, respiratory pump, and humidifier for inspired air. Figures 1, 2, and 3 are photographs of the respirator assembly. The respirator consisted of a Lucite cylinder, 6.5 in. in diameter and 21 in. long. A 6-in. extension to the cylinder was used for larger animals. A rubber collar fitted snugly around the monkey's neck. The inner section of the collar was gum rubber that stretched when slipped over the animal's head. A metal plate held the collar in place at one end of the cylinder. Plastic slides and a head holder to support the neck were held in place with stud bolts in the end of the cylinder. On the other end of the cylinder was a plastic end plate with two small holes. A metal stopcock was applied to one hole and a hose connection to the other. Plastic guards were placed over the holes on the inner side to prevent the animal from plugging the opening with its feet. A vacuum pressure gage on the side of the cylinder showed the pressure changes within the cylinder during a respiratory cycle when the respirator pump was in operation. The respirators, which were set in cradles, could be rotated from one side to the other (approximately 150°) by hand.

The platform supporting the respirator was hinged to the front edge of a table so that the platform could be tilted to elevate the posterior end of the respirator as much as 60°. This was done to prevent the excessive secretions formed in the respiratory tract from flowing into the small bronchial tubes and to aid in the removal of the secretions.

A Harvard respirator pump, model No. 607, was used to create negative pressure in the cylinder, varying between approximately 3 and 18 cm ($H_2O$). The rate of respiration was usually set at 20/min.

To prevent excessive drying of the secretions in the respiratory tract during AR, the humidity of the inspired air was increased. A copper tube, 1 in. in diameter and approximately 30 in. long, was attached to a 2-*l* bottle of water set in a large water bath that was kept at approximately 50°C. Air was blown over the surface of the water in the 2-*l* bottle, through the copper tube connected to a plastic tube, and into a funnel-shaped cup over the face of the animal (figure 2). This arrangement supplied air with a high humidity at room temperature to an endotracheal tube.

5. Resuscitator (An Automatic Positive-Pressure Type for Giving Oxygen Through an Endotracheal Tube). The AVR (anesthetizer, vaporizer, and resuscitator) apparatus made by National Cylinder Gas, Division of Chemetron Corporation, was used when the animal was removed from the respirator and still needed AR. During its operation, oxygen from a gas cylinder was passed through the AVR, which produced a complete cycle of respiration.

FIGURE 1

SIDE VIEW OF RESPIRATORS

(One shows monkey in position, other shows components of head holder)

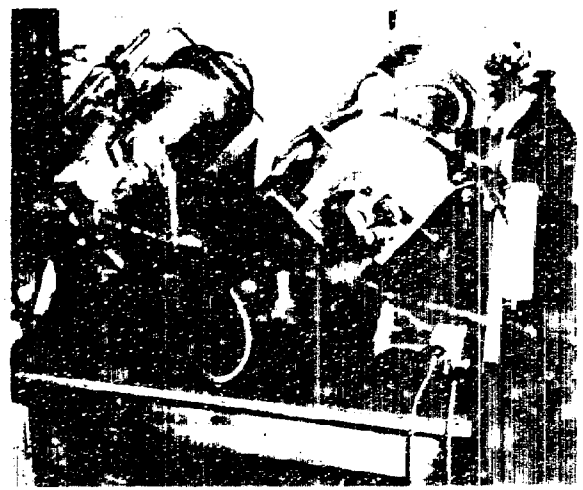

FIGURE 2

FRONT VIEW OF RESPIRATORS

(Monkeys are shown in position)

FIGURE 3

REAR VIEW OF RESPIRATORS

(Pumps are under table, and water bath with humidifier is on floor)

It automatically adjusted the volume of inspired oxygen to the size of the animal's lungs, rhythmically and automatically filling the lungs to 13 mm Hg pressure and causing exhalation at 9 mm negative peak pressure.

B. Procedure.

Monkeys received the botulinum toxin iv in the following doses: 5, 12, and 24 LD50's (1 LD50 for the rhesus monkey was reported by Herrero and coworkers[2] to be 40 MU/kg). Times of occurrence for selected toxic signs (ptosis of the eyelid, general weakness, and death) were recorded.

A group of nine untreated animals served as controls. A group of four monkeys received only AR. Another group of six received both antitoxin and AR. The antitoxin (1,000 to 2,700 units per animal) was given iv when the toxic signs were severe and some difficulty in breathing was noted. (One animal, No. 5, collapsed and was immediately placed in the respirator before antitoxin administration, which, in this case, was into a vein under the tongue.) AR was initiated when the animal was in severe respiratory distress and was near collapse. To reduce struggling of the animals while they were being placed in the respirator and to facilitate inserting the endotracheal tube to maintain an airway during AR, a small dose of a short-acting thiobarbiturate, such as Pentothal Sodium, Surital, or Kemithal, was administered iv.

After the animal was placed in the respirator, the assembly usually was rolled from one side to the other every 2 to 4 hr to reduce hypostatic congestion of the animal's lungs. Furthermore, in most of the tests, the respirator was tilted about 35° to lower the head so that tracheal drainage would be away from the lungs. When animals were removed from the respirator for feeding, oxygen was supplied through the endotracheal tube by the AVR.

Most of the treated animals received some supportive treatment, usually beginning about 48 hr after administration of the toxin, which consisted either of ig feedings (milk mix), glucose (5%) ip, or saline iv, or combinations of these and a vitamin supplement. An antibiotic (Combiotic) was also injected at this time.

Since there were no survivors, extension of time to death over that for the untreated controls was taken as a measure of therapeutic effectiveness. After an animal died, gross examination of the trachea and lungs was made in some instances. Gross and microscopic examinations were made in greater detail on a few animals.

## III. RESULTS.

### A. No Treatment (Controls).

In the table are data on the times to occurrence of two prominent early toxic signs and times to death of untreated monkeys after iv injections of the toxin. Regardless of the dose, toxic signs were seldom seen before 20 hr. Usually ptosis, yawning, and abnormal head positions were the earliest signs, and these were followed by generalized weakness, increased salivation, and drooling (figures 4 and 5). Dyspnea, head and shoulders drooping forward, and collapse usually occurred several hours prior to death. All of the animals died. The larger doses usually resulted in earlier onset of toxic signs and death.

### B. Respirator Treatment With and Without Antitoxin.

The table shows a comparison of time to death after AR, AR plus antitoxin, and no treatment. The average times to death are taken as a measure of the effectiveness of treatment in the dosage levels studied. In all instances, the treated animals lived longer than the controls; this difference is statistically significant. The average survival time for the AR-treated animals (no antitoxin), though limited in number, appears to be longer than the average for the antitoxin-treated animals; however, this difference is not statistically significant.

From the subjective point of view, the antitoxin-treated animals always appeared to be more alert than those not receiving antitoxin. The AR-treated animals (no antitoxin) alternated between a state of alertness and coma, and they were less responsive to external stimuli during the last 24 hr prior to death. Deaths were caused by botulism or pulmonary complications resulting from prolonged AR.

The clinical courses of animals No. 46 and 53 are described in some detail because these monkeys were the only ones to develop spontaneous respiration.

Animal No. 46 (5.0 LD50's of toxin) had antitoxin, nursing care, drugs, and iv feedings. After the animal had been in the respirator for 33.5 hr (67 hr postexposure), the pump was stopped for a moment to determine whether the animal would make any spontaneous effort to breathe. It was able to breathe, but with some difficulty. The air inhaled by the animal was enriched with oxygen. After 3.5 hr, AR was resumed for another 5 hr, after which the animal was transferred to an oxygen tent. The animal was able to stand up in the tent and move around, but was weak and quivering. At times,

its head dropped forward. A gasping type of respiration, with considerable drooling, was observed. After 6.3 hr in the tent, the animal collapsed and died. Necropsy revealed a consolidated lung that possibly prevented oxygen transfer from the lungs to the blood. Details of treatment of this animal are given in the appendix.

Animal No. 53 (5.0 LD50's of toxin) received antitoxin and adequate nursing care. After 16 hr (36.5 hr postexposure), the respirator was stopped; the animal was able to breathe spontaneously, but with difficulty. The animal was not removed from the respirator, but was supplied with oxygen or oxygen-enriched air through the endotracheal tube for 6.3 hr. It also received nourishment by stomach tube. When breathing appeared to be labored and of a gasping type, oxygen was administered by AVR for 1 hr. After this, the animal again breathed spontaneously and received oxygen-enriched air for 0.7 hr and additional feedings. This was followed by a 7.3-hr interval of oxygen by AVR, after which the animal was placed in an oxygen chamber and allowed to breathe spontaneously for 13.2 hr. During this period, the animal was weak and lay on its side most of the time. When disturbed, however, it could rise, stand on its feet, and fight with considerable vigor. Its mouth was pink, indicating that sufficient oxygen was supplied to the blood. On auscultation of the chest, the flow of air through the trachea sounded normal. The heart also sounded normal, the beats being strong and regular. The respiration rate was normal, although breathing was largely diaphragmatic. After the animal had spent nearly 12 hr in the chamber, breathing became labored, and it was feared that death might ensue at any moment because of respiratory failure. The monkey was then removed from the chamber and given oxygen through a tracheal tube by means of the AVR. Another iv feeding was administered. Some improvement was noted, as reflected by the color of the mouth and positive palpebral response.

The AVR was stopped temporarily to see whether spontaneous respiration would be resumed. Respiration was inadequate, the animal becoming cyanotic and unconscious. Again AVR was resumed for nearly an hour. The color of the mucous membranes of the mouth indicated that AR was still inadequate; therefore, the AR was discontinued. The spontaneous respiration at this time was irregular and of a gasping type. The dying animal failed to respond to mechanical stimulation. Death occurred 78.3 hr after administration of the toxin.

Upon necropsy, 15 gm of thick, reddish, mucuslike plugs were removed from the main-stem bronchi. The surface of the lungs appeared to be normal, except for some small, dark areas on both lobes. A cut section of one lobe showed small areas of congestion. A thick, whitish substance was pressed from the tissues. Death was attributed to pulmonary obstruction.

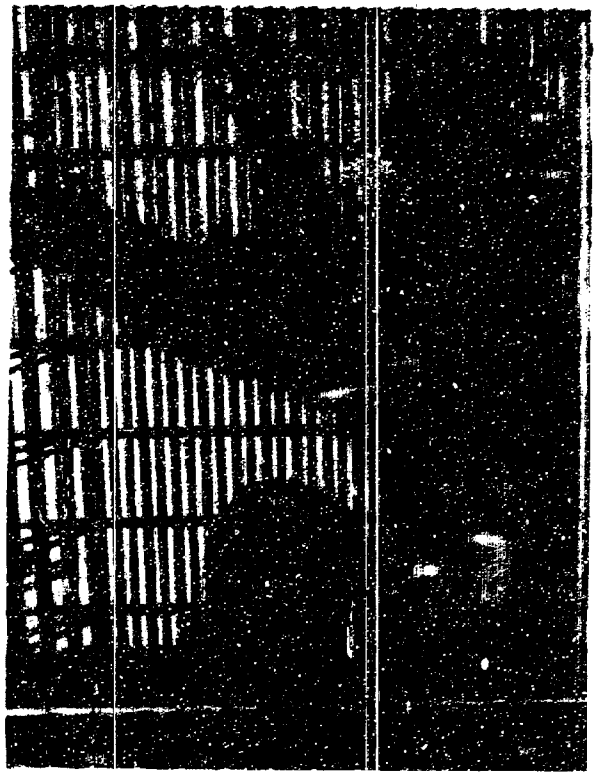

FIGURE 4

EARLY STAGE OF BOTULISM, SHOWING PTOSIS AND WEAKNESS

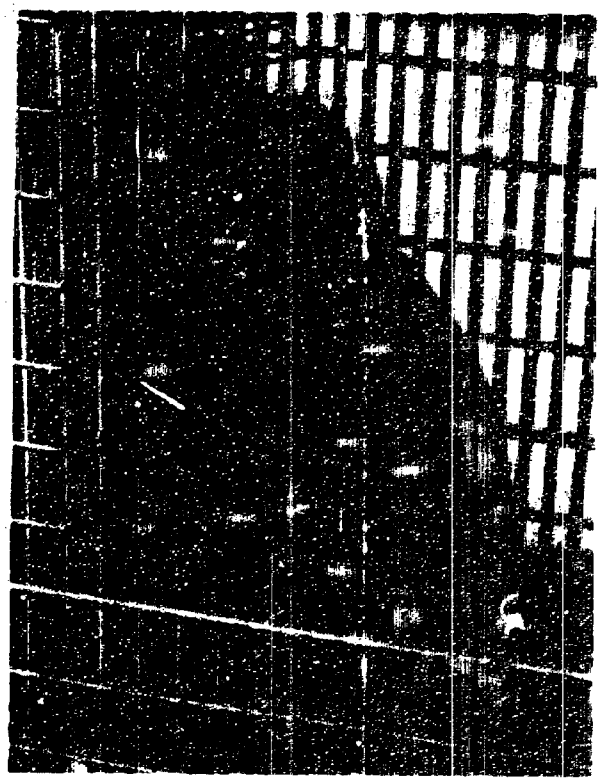

FIGURE 5

INCREASED TOXIC EFFECTS

TABLE

EFFECT OF AR AND AR PLUS ANTITOXIN IN MONKEYS WITH RESPIRATORY PARALYSIS FROM BOTULINUM TOXIN

| Animal number | LD50's of toxin | Appearance of mild toxic signs: ptosis and muscular weakness | Time to anti-toxin adminis-tration | Time to AR | Duration of AR and SR* periods | | | | | | | | | | Time to death | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | AR | SR | AR | SR | AR | SR | AR | SR | AR | SR | | |
| | | | hr | | hr | | | | | | | | | | hr | |
| **A. No Treatment** | | | | | | | | | | | | | | | | |
| 1 | 24 | 21.5 | | | | | | | | | | | | | 28 | |
| 11 | 24 | 16.5 | | | | | | | | | | | | | 33 | |
| 24 | 24 | – | | | | | | | | | | | | | 17 | |
| 57 | 12 | – | | | | | | | | | | | | | 20 | |
| 45 | 5 | – | | | | | | | | | | | | | 43 | |
| 51 | 5 | 28 | | | | | | | | | | | | | 64 | Average |
| 60 | 5 | 28.6 | | | | | | | | | | | | | 49 | 53 |
| 64 | 5 | 34.4 | | | | | | | | | | | | | 73 | |
| 17 | 5 | 29 | | | | | | | | | | | | | 38 | |
| **B. Treated With AR** | | | | | | | | | | | | | | | | |
| 8 | 24 | 42.0 | | 45.2 | 7.8 | | | | | | | | | | 53.0 | |
| 50 | 5 | 25.0 | | 31.5 | 65.8 | | | | | | | | | | 97.3 | Average |
| 62 | 5 | 25.1 | | 30.1 | 147.3 | | | | | | | | | | 177.4 | 116 |
| 63 | 5 | 28.3 | | 35.0 | 39.0 | | | | | | | | | | 74 | |
| **C. Treated With Antitoxin and AR** | | | | | | | | | | | | | | | | |
| 5 | 24 | 20.5 | 22.6 | 21.5 | 53.5 | | | | | | | | | | 75 | |
| 43 | 12 | 18.9 | 19.2 | 21.7 | 62.3 | | | | | | | | | | 84 | |
| 46 | 5 | 33.5 | 33.5 | 33.5 | 33.5 | 2.5 | 4.6 | 6.7 | | | | | | | 80.8 | |
| 48 | 5 | 25 | 25.3 | 30.3 | 54.7 | | | | | | | | | | 65 | Average |
| 53 | 5 | 26.3 | 30.5 | 30.5 | 16.0 | 6.3 | 1.0 | 0.7 | 7.3 | 13.2 | 1.3 | 0.1 | 0.9 | 1.0 | 78.3 | 89 |
| 65 | 5 | 30.8 | 38.8 | 39.0 | 94 | | | | | | | | | | 133 | |

* Spontaneous respiration.

Descriptions of some other treated animals, in which no technical difficulties were encountered in AR, are given in the appendix. Post-mortem examinations of some animals are included.

A review of all AR-treated animals that were necropsied showed that most of those that survived as long as 48 hr after the toxin had little or no lung-tissue damage. Those surviving longer usually did have lung involvement, which included lung consolidation, bronchopneumonia, or pulmonary atelectasis. Cellular infiltrates of lymphocytes, polymorphonuclear leucocytes, and eosinophils were also noted. The bronchial submucosa was edematous and hypersecretory.

## IV. DISCUSSION.

There is ample information available in the literature showing that botulinum antitoxin will prevent death when administered soon after the toxin and long before the appearance of toxic signs. In mice receiving 40 to 80 LD50's of toxin, the critical time for administration of antitoxin is a short time before any toxic signs appear.* It is known that antitoxin injected into the bloodstream of dogs neutralizes all the uncombined toxin in the circulation.[3] At present, there is no evidence that the portion of the toxin fixed by the tissues is affected by antitoxin. In another study,[4] it was demonstrated that at least 80% of monkeys given toxin iv in doses as high as 5 LD50's can be saved, provided the antitoxin is administered soon after the appearance of the first signs and that suitable nursing care is provided during the period of aphagia. Under these conditions, AR is usually not necessary; however, if the toxic signs are severe when antitoxin is given, then AR in addition to antitoxin may be of value in prolonging life. Such treatment, supplemented by ig feeding, could be lifesaving, provided that the air passages of the lungs have not become severely obstructed with mucuslike debris or the lungs have not become congested or consolidated as a result of prolonged AR. In the studies on monkeys showing severe toxic signs before treatment, AR and antitoxin were not sufficiently effective to cause permanent recovery of any animal, possibly because of congestion or consolidation of the lungs, or both.

During the course of this study, 16 additional animals were treated in the manner described above with AR and antitoxin and 3 with AR and no antitoxin. These 19 animals are not included in the table. They were excluded because of various accidental technical difficulties encountered during the AR period, and the animals died from suffocation. These accidents usually occurred when no attendant was present. In some instances, the tracheal tube slipped out of the trachea or the tracheal tube become plugged with dried,

* Crook, J. W., Cresthull, P., and Oberst, F. W. The Effectiveness of Bivalent Botulinum Antitoxin and Drug Therapy Against Clostridium Botulinum Type A Toxin in Mice. (In preparation.)

mucuslike material. In several other instances, the respirator developed a leak at the seal around the neck, and the animal did not respire. One animal actually showed initial signs of spontaneous respiration, being able to move its arms and legs and to make several feeble efforts to breathe, before the accident occurred. The averages of the times to death for these two groups of animals were significantly longer than those for the control group. Had the accident not occurred, the lifespan for each animal probably would have been longer. For 10 of these animals, deaths occurred between 41 and 76 hr after toxin.

A significant finding in this study was that temporary periods of spontaneous respiration occurred in two animals receiving antitoxin and AR after 5 LD50's of toxin. Spontaneous respiration, though not entirely satisfactory, was maintained until the gaseous exchange in the lungs was inhibited by lung consolidation or plugging of airway passages. It is possible that complete recovery would have occurred if the lungs had been normal and capable of transporting oxygen to the blood. Animals not reported in this study that died from experimental mishaps 36 to 48 hr after injection of toxin usually had relatively normal-appearing lungs. Usually, little or no tracheal debris was seen. Gross and microscopic examination of the lungs of animals surviving 60 hr or more showed considerable congestion and consolidation. Some had confluent pneumonia. Usually, mucuslike debris was present in the trachea. Tracheitis was seen in some animals. Herrero and associates[2] reported that they did not see these conditions in their untreated monkeys that lived longer than 60 hr after iv toxin. It is believed, therefore, that the lung conditions seen after AR are the result of mechanical trauma from AR and irritation from the tracheal cannula.

After examination of a number of lungs on necropsy, it was realized that treatment must be directed to the prevention of plugging of airways and consolidation of the lungs during AR. Since viscid, tenacious mucus was found in the trachea and in the airway passages of the lungs, attention was paid to the removal of this material from the trachea and to the prevention of its entrance into the bronchi and lungs. Attempts to aspirate the viscous material were not fruitful; usually, only a small amount could be removed. Necropsy revealed that this material was spread on the tracheal wall in a thin layer.

Another technique for preventing the tracheal exudate from getting into the lungs was to elevate the posterior end of the respirator so that the head of the animal was lower than the lungs, thereby enabling some of the fluid in the trachea to drain away from the lungs. Actually, this position was more desirable for aspiration of the fluid, since some drained out of the endotracheal tube in one animal when the face was turned down. To prevent hypostatic congestion and lung consolidation caused by pooling of blood in the

underside of the lung, the animal was frequently turned from side to side. This procedure was of considerable importance. There was one animal, not reported in the table, that was not turned in 30 hr; the entire underside of the lung was almost black and completely consolidated.

It may be of interest to compare the results of therapy on monkeys in these tests with that of a patient with botulism who received essentially similar therapy. In two papers, Tyler[5,6] reported his findings in a 64-yr-old patient with botulism. He treated this patient symptomatically, also giving 16,000 units of polyvalent (Types A and B) botulinum antitoxin (Lederle) iv and AR (Bird respirator). After 5 days, the patient did not respond to resuscitative measures and died. Since neither of Tyler's papers contained a report from the autopsy, information was obtained directly from the author through the Department of Pathology, Peter Bent Brigham Hospital. The following statements are in the pathology report: "At autopsy an acute tracheobronchitis and marked confluent bronchopneumonia affecting mainly the left upper and to a lesser extent the right upper and left lower lobes, was found . . . The severe pneumonia was considered to be the immediate cause of death." Organ weights were also given in the report. The lungs weighed 60% more than the average value for normal adults. This increase in weight was probably due to the consolidation and congestion of the lungs.

The results of therapy during prolonged AR in this patient are quite similar to those in the monkeys used in this study. The main similarity is in the lung pathology after prolonged AR. Because of the severe congestion in the lungs or the plugging of bronchioles, the gaseous exchange in the lungs becomes inadequate. This inadequacy resulted in cardiac difficulties and eventually in death.

Tyler also reported that his patient had decreased lacrimation and salivation. There was a dryness of the mouth. The sclera and corneas were very dry, but sweating was normal. No mention was made of a thick mucoid discharge being present in the mouth and paranasal sinuses, as was seen in some of the monkeys at these Laboratories. In some animals, the mouth became full of the discharge because the animal usually was unable to swallow. This resulted in drooling; some animals would take their hands and pull some of the ropy material as strings from the mouth. Methylene blue crystals were placed in the mouth of one animal. The animal was killed an hour later, and the esophagus and trachea were examined for the presence of methylene blue; none was found. A normal monkey, not given toxin, also received methylene blue. In this instance, the dye disappeared from the mouth, but was found in the esophagus and stomach; none was found in the trachea.

The finding of a considerable amount of thick, mucoid debris in the trachea of some animals is of concern. It is possible that some of this could have come from the oral cavity, but this is not likely because no dye was found in the trachea. It is more likely that the material is secreted in the mucosa of the trachea from the many goblet cells and mixed seromucus glands. A large number of these is also present in the mucosa of the sinuses and may be the source of the thick, tenacious mucus seen in the mouth. The salivary glands should not be ignored because they, too, contain mucus-producing cells, but the secretion from these usually does not have the characteristics described.

## V. CONCLUSIONS.

In monkeys with respiratory paralysis from lethal doses of botulinum toxin, AR, with and without antitoxin, aids in prolonging life; however, continued long-term treatment may result in irreversible lung damage, probably caused by the method of AR.

## LITERATURE CITED

1. Lamanna, C., McElroy, O. E., and Eklund, H. W. The Purification and Crystallization of Clostridium Botulinum Type A Toxin. Science 13, 613, 614 (1946).

2. Herrero, B. A., Ecklund, A. E., Streett, C. S., Ford, D. F., and King, J. K. CRDLR 3235. Experimental Botulism in Monkeys—A Clinical Pathological Study. 1964. UNCLASSIFIED Report.

3. House, M. J., Cresthull, P., Crook, J. W., and Oberst, F. W. CRDLR 3229. Changes in Concentration of Botulinum Toxin in Dog Serum After Parenteral Administration. 1964. UNCLASSIFIED Report.

4. Oberst, F. W., Cresthull, P., Crook, J. W., and House, M. J. CRDLR 3331. Botulinum Antitoxin as a Therapeutic Agent in Monkeys After Experimental Botulism. 1965. UNCLASSIFIED Report.

5. Tyler, H. R. Botulism. Arch. Neurol. 9, 652-660 (1963).

6. Tyler, H. R. Physiological Observations in Human Botulism. Ibid., 661-670 (1963).

# APPENDIX

## DETAILED COURSE OF TREATMENT

### I. EXPLANATORY NOTES.

AT = Botulinum antitoxin, always administered iv.

AR = Artificial respiration with a Drinker-type respirator.

SR = Spontaneous respiration.

AVR = Resuscitator (anesthetizer, vaporizer, and resuscitator) capable of automatically filling the animal's lungs with oxygen and exhaling the used gases in perfect rhythm. It is made by National Cylinder Gas, Division of Chemetron Corporation, 840 N. Michigan Avenue, Chicago 11, Illinois.

Ig feeding = Fluid injected through a tube into the stomach.

Milk mix = Sobee Powder Milk Mix in $H_2O$ according to directions. Sobee Powder is made by Mead Johnson and Company, Evansville 21, Indiana. In normal dilution (w/v), it contains the following ingredients: protein, 3.2%; fat, 2.6%; carbohydrate, 7.7%; crude fiber, 0.2%; minerals (ash), 0.5% including calcium, 0.1%; phosphorus, 0.05%; iron, 0.0005%; and moisture, 85.8%; 100 gm supplies 465 calories.

Kemithal = Thialbarbitone: sodium 5-allyl-5-(2-cyclohexenyl)-2-thiobarbiturate.

Surital Sodium = This is an ultra-short-acting anesthetic made by Parke, Davis. It is thiamylal sodium [5-allyl-5-(1-methylbutyl)-2-thiobarbiturate].

Combiotic = Each 2 ml contains 400,000 units penicillin G procaine crystalline and 0.5 gm dihydrostreptomycin as the sulfate with 2% procaine HCl and preservatives. Pfizer Laboratories, Division of Charles Pfizer and Company, Inc., New York, New York.

Eltradd-1000 = A balanced electrolyte powder for preparing ig solutions for animals. It contains the electrolyte ions sodium, potassium, calcium, magnesium, chloride, and bicarbonate (after metabolic conversion) and the trace elements cobalt, zinc, manganese, copper, iodine, and iron. Haver-Lockhart Labs, Kansas City, Missouri.

Amino-Plus = This product is made by Pitman-Moore. It is a new amino acid, dextrose, and vitamin B powder. By adding a suitable diluent, a solution designed for parenteral use is prepared. Each 250-ml vial (restored) contains 10 gm amino acids, 10 gm dextrose, plus 83.5 mg thiamine hydrochloride, 8.5 mg riboflavin, 8.5 mg pyridoxine hydrochloride, 66.5 mg calcium pantothenate, and 333.0 mg nicotinamide with 25 mg thimerosal (preservative).

## II. ARTIFICIAL RESPIRATION (NO ANTITOXIN).

### A. Animal No. 50.

This animal received 5.0 LD50's of toxin, and the times in hours to effects after toxin administration are as follows:

25.0 Ptosis
30.5 Head drooping
31.5 Surital, iv (31 ml); AR
45.0 Profuse thick mucus from mouth and nose
48.0 Aspirated trachea
48.7 $O_2$ by AVR; Combiotic, im (2 ml); saline, iv (60 ml)
49.0 Back on respirator
52.0 Aspirated trachea; turned body to opposite side
54.0 Aspirated; turned body stomach-down
64.0 Saline, ip (100 ml)
69.8 $O_2$ by AVR; milk mix, ig (50 ml, 80 cal); dextrose, 50%, iv (5 ml); saline, ip (100 ml); Combiotic, im (2 ml)
89.7 Back on respirator
90.5 Amino-Plus, ip (15 ml); saline, iv (100 ml)
91.5 Removed from respirator
91.7 $O_2$ through tracheal tube by AVR
92.0 Armine, 1%, im (0.1 ml) : 1 ml saline; dextrose, 50%, iv (5 ml)
94.0 Armine, 1%, im (0.2 ml): milk mix, ig (50 ml)
95.5 Returned animal to respirator for AR
97.3 Died (sacrificed)

During the last 18 to 24 hr, animal No. 50 periodically appeared to be unconscious, as no response was evoked by touching its eyelids. The heart rate was slower than normal, and, on auscultation, the heart sounds were sharp and distinct. This animal had received considerable nursing care and supportive treatment, such as antibiotics, dextrose, ig feeding, frequent turning of the respirator, etc. After 97 hr, it was thought that death would

occur any moment, so the chest of the animal was opened. The heart continued to beat slowly and responded to an intracardiac injection of epinephrine; the force of contractions increased markedly within a minute. On necropsy, the trachea was found to contain small pieces of fibrinlike clots, although the bronchi were free of mucuslike plugs. The uncut surface of the lungs appeared reasonably normal, except for small areas of congestion.

B. Animal No. 62.

This animal received 5.0 LD50's of toxin, and the times in hours to effects after toxin administration are as follows:

| | |
|---|---|
| 25.1 | First toxic signs |
| 30.1 | Surital, iv (30 ml); placed in respirator, tilted respirator periodically; aspirated fluids from trachea periodically |
| 48.5 | Dextrose, 5%, ip (200 ml); Combiotic, im (1.25 ml) |
| 51.4 | Loss of eye reflex, color pale; $O_2$ given for 2 min by AVR; turned face down occasionally to drain secretions from mouth; eye reflex restored after $O_2$ administration; mouth is pink |
| 66.0 | Respirator stopped momentarily; no SR |
| 67.0 | Powdered-milk mix,ig (100 ml) |
| 68.0 | Dextrose, 5%, ip (200 ml); positive pressure $O_2$ (12 min) |
| 72.0 | Loss of eye reflex; cleaned endotracheal tube; $O_2$ given for 8 min until eye reflex returned |
| 85.0 | Cleaned endotracheal tube |
| 96.0 | Powdered-milk mix, ig (100 ml) plus Octavitamin (1 ml); dextrose, 5%, ip (200 ml); $O_2$ given for 5 min |
| 99.0 | Monkey rolls eyes, blinks, and can see; responds to stimuli |
| 114.0 | Animal rolls eyes; no body movements |
| 123.0 | $O_2$ by AVR; powdered-milk mix (100 ml) plus Octavitamin (1ml), ig; dextrose, 5%, ip (200 ml) |
| 124.0 | Endotracheal tube cleaned |
| 148.0 | $O_2$ by AVR for 5 min; powdered-milk mix (100 ml) plus Octavitamin (1 ml), ig; dextrose, ip (200 ml) |
| 157.0 | Eyes roll freely, mouth and lips move |
| 163.0 | Changed endotracheal tube |
| 168.0 | Monkey removed from respirator and given bath; $O_2$ given by AVR |
| 170.0 | Color of mouth and tongue good; pupil and eye reflex normal; Dextran, ip (290 ml); powdered-milk mix, ig (100 ml) plus Octavitamin (1 ml); monkey back in respirator |
| 170.2 | Loss of eye reflex; hypoxic; aspirated and removed dried blood clot from airway; reflex returned and color good; does not blink spontaneously |

170.9 Blinks spontaneously
171.1 Does not blink or roll eyes; color good
171.9 Aspirated fluids appear bloody
172.1 No air movement in tracheal tube; rinsed tracheal tube with a few milliliters of $H_2O$
172.2 Aspirated blood clots; $O_2$ by AVR; eye reflex returned
172.4 Air moving in endotracheal tube; eye reflex absent; $O_2$ by AVR discontinued; respirator turned on
173.7 Eyes move spontaneously; blinks in response to blowing air on eyes; body beginning to get cold and eye reflex disappearing
177.2 Animal in shock, pale, cold
177.4 Dead

This animal received better care after the toxin than any other animal studied. It lived longer than any other AR-treated animal. The monkey was regularly turned from side to side. It received fluids, liquid food, vitamins, and antibiotics during AR. Whenever the animal appeared to become comatose because of plugging of the endotracheal tube, removal of the obstruction and administration of oxygen restored consciousness. The trachea was aspirated frequently to remove mucus and other debris. During the last 24 hr, the animal gradually weakened; mouth and eye movements gradually disappeared. Body temperature became subnormal, and the animal passed into a state of shock and died.

1. Internal Examination.

There were focal, depressed, deep-red areas in the posterior segments of the lungs. The trachea was moderately hyperemic. There was a 1-cm, gritty, pultaceous, yellow, partly calcified mass partially adherent to the right adrenal gland. The endocardium of the left ventricular outflow tract was focally ecchymotic.

2. Microscopic Examination.

a. Lung: Multiple sections of lung show normal alveoli and distal air spaces. The bronchial mucosa shows foci of squamous metaplasia and of reserve cell hyperplasia. These are changes caused by chronic irritation and are unrelated to the toxin.

b. Trachea: Focal mucosal hemorrhage.

c. Ovary: There is a corpus luteum in the stage of cascularization.

d. Uterus: The endometrium is in midsecretory phase.

e. Liver: Normal.

f. Kidney: Normal; no inclusions in the pelvis.

g. Adrenal: There is an old granuloma containing dead parasitic ova adherent to the right adrenal, but not involving it. Many cortical cells have large eosinophilic cytoplasmic inclusions, sometimes several to a cell. Are these a simian virus?

3. Final Anatomical Diagnosis.

a. History of botulism.

b. Focal atelectasis of the lungs.

c. Squamous metaplasia of the bronchial mucosa.

d. Old parasitic granuloma, right retroperitoneal soft tissue.

e. Eosinophilic cytoplasmic inclusions in the adrenal cortex.

C. Animal No. 63.

This animal received 5.0 LD50's of toxin, and the times in hours to effects after toxin administration are as follows:

28.3 First toxic sign appeared: slight ptosis
35.0 Surital, iv (2 ml); placed in respirator, periodically turned from side to side, and aspirated trachea
48.0 Alert; blinks eyes
51.0 $O_2$; dextrose, 5%, ip (200 ml)
72.7 Powdered-milk mix, ig (100 ml) with Octavitamin (1 ml)
73.2 Dextrose, 5%, ip (300 ml)
73.7 Pupils fail to respond to light; monkey appears dead; $O_2$ by AVR; no response to $O_2$; respirator turned on again
74.0 No heart sound (dead); removed from respirator

Animal No. 63 also received excellent nursing care, although it survived only 74 hr.

1. Internal Examination.

Patchy areas of atelectasis in lungs. No other lesions noted.

2. Microscopic Examination.

a. Kidney: Focal chronic inflammatory cells in a few of the interstitial areas of the cortex; inclusion bodies are seen in the renal pelvis.

b. Bladder, uterus, stomach, liver, skeletal muscle, and heart: All normal.

c. Ovary: Many follicles present with one corpus.

d. Intestine. Focal areas of chronic inflammatory cells in lamina propria.

e. Lung: Areas of atelectasis, cellular infiltrates of lymphocytes, polymorphonuclear leucocytes (PMN's), and eosinophils particularly evident peribronchially. Much dark, brownish-black pigment is noted throughout. Lung mites are noted in several cross sections of the lungs. Emphysematous areas are noted in several sections. In some bronchi, in addition to the mites, there are red blood cells, edema fluid, and neutrophils. Bronchial submucosa are edematous and hypersecretory.

f. Bronchial lymph node: Contains much dark, brownish-black pigment.

g. Trachea: Patches of the epithelial lining have undergone squamous metaplasia. Some of the submucosa show hemorrhage and inflammatory cells infiltrating.

3. Final Anatomical Diagnosis:

a. Bronchopneumonia.

b. Pulmonary acariasis.

c. Tracheitis.

d. Inclusion bodies in renal pelvis.

e. Death of this animal could, at least in part, be caused by the bronchopneumonia that had developed.

## III. ANTITOXIN AND ARTIFICIAL RESPIRATION.

### A. Animal No. 5.

This animal received 24 LD50's of toxin, and the times in hours to effects after toxin administration are as follows:

20.5 Ptosis, head drooping, salivation, gasping type of respiration
21.5 Very weak; AR
22.0 $O_2$ plus air mixture by inhalation; Prostigmine, sc (0.01 mg/kg)
22.3 Endotracheal tube inserted; $O_2$ administered
22.6 AT (2,000 u); Prostigmine, im (0.004 mg/kg)
22.8 $O_2$ discontinued
24.6 Alert, blinks eyes, color good, unable to breathe without respirator; Prostigmine, im (0.004 mg/kg)
27.0 Same as above
29.9 Prostigmine, sc (0.004 mg/kg)
31.0 Alert
31.7 Alert, attempts to bite when touched on face
33.2 Alert, has strong bite; turned position of monkey to clear fluid from lungs
33.4 Active, moves arms and legs; pupils normal size, respond to light; turned monkey back to original position
33.8 Same as above
35.5 Prostigmine, sc (0.004 mg/kg)
45.8 Milk mix, ig (150 cal); Prostigmine, sc (0.004 mg/kg)
46.0 Removed from respirator; $O_2$ given by endotracheal tube using AVR
47.0 Prostigmine, im (0.002 mg/kg)
47.3 Returned to respirator
49.5 Prostigmine, sc (0.004 mg/kg)
52.0 Prostigmine, sc (0.004 mg/kg)
52.2 Milk mix in 20 ml saline and 120 ml $H_2O$ (250 cal)
71.3 Heart sound not as strong as on previous day, harsh rales, cracking and popping sounds in chest and tracheal tube; removed from respirator; penicillin procaine G, im (300,000 u); atropine, im (0.14 mg/kg); Prostigmine, im (0.004 mg/kg); milk mix, ig (300 cal) plus 20 ml tap water; $O_2$ given by AVR
71.5 Returned to respirator; aspiration of fluid and thick reddish mucus from endotracheal tube; $O_2$ given through endotracheal tube
71.7 Not responding as well, dazed, eye reflexes poor
73.5 Animal begins to roll eyes, pupils respond to light
74.0 Dying
75.0 Dead (congestion of trachea and lungs)

Monkey No. 5 received 24 LD50's of toxin. In addition to AT and AR, this animal was given liquid food by stomach tube several times and also various drugs parenterally. Prostigmine was injected a number of times. From the results in this animal and from those in other Prostigmine-treated

animals, it is doubtful that it was of any value as a therapeutic agent. This animal did not develop any signs of spontaneous breathing during AR. As death approached, rales and cracking noises were heard in the endotracheal tube, indicating the presence of fluid in the trachea. Some of this fluid was aspirated through a small tube inserted through the endotracheal tube. Only a small amount of thick, sticky, mucuslike, reddish material could be withdrawn. Perhaps this material plugged some of the bronchi and contributed to the lung congestion that was revealed by necropsy. In this animal, AR was of little value even when oxygen was supplied.

On necropsy, the trachea contained a reddish, thick, mucuslike material, some of which extended into the bronchial tree. The trachea appeared to be inflamed. On the surface of the lungs were large, dark-red areas suggesting possible congestion in the tissues. Emphysema was also seen. Microscopic examination showed acute inflammation in the mucosa of the trachea. There were patchy areas of severe, acute pulmonary edema, congestion, and hemorrhage in the lung tissues. There was ample evidence that death was due to respiratory insufficiency secondary to the intoxication by the toxin.

1. Gross Examination.

a. Intestine, stomach, diaphragm, kidney, liver, adrenals, and esophagus: Normal.

b. Spleen: Normal, except for small size.

c. Right lung: Intermediate lobe diffusely red throughout parenchyma; some emphysema.

d. Left lung: Intermediate lobe same as right one; diaphragmatic lobes adhered to diaphragm by fibrin; alveolar spaces and bronchial tree contained serous fluids with red cells and small amounts of mucuslike material.

2. Microscopic Examination.

a. Lung: Patchy areas of severe pulmonary congestion, edema, and frank hemorrhage; aspirated bloody material seen in the bronchi.

b. Trachea: Acute inflammation of the mucosa.

c. Liver: Normal.

d. Diaphragm: Normal.

3. Final Anatomical Diagnosis.

a. Acute tracheitis.

b. Acute pulmonary edema, congestion, and hemorrhage.

c. Aspiration of bloody material in bronchi.

4. Probable Cause of Death.

Respiratory insufficiency secondary to toxin.

B. Animal No. 43.

This animal received 12 LD50's of toxin, and the times in hours to effects after toxin administration are as follows:

18.9 Salivation, foamy mouth, severe ptosis, weak
19.2 AT (1,000 u)
21.6 AT (1,000 u)
21.7 AR
21.9 Endotracheal tube inserted; $O_2$-enriched air (2 hr)
44.0 Dextrose, iv (75 ml; then by slow drip, 350 ml); benzathine penicillin G suspension, im (2 ml, 1,200,000 u)
84.0 Dead (lung congestion)

Animal No. 43 was treated somewhat similarly to No. 5, except that no Prostigmine was administered. Necropsy showed that death was caused by suffocation from occluded bronchi.

1. Gross Examination.

a. Right lung: Severe hemorrhagic congestion including entire right lung; early stage of pneumonia that probably developed during the last 2 to 3 days prior to death.

b. Left lung: Diffuse pneumonia in early stage.

c. Trachea: Excess secretion in distal end and in both bronchi.

2. Cause of death.

Hemorrhagic congestion; accumulation of fluids in trachea; occlusion of bronchi.

C. Animal No. 46.

This animal received 5 LD50's of toxin, and the times in hours to effects after toxin administration are as follows:

33.5 Ptosis, weak; AT (2,000 u); thiobarbiturate; AR
44.3 $O_2$ through tracheal tube; saline, ip (100 ml); digoxin (0.13 mg)
51.0 Glucose, ip (100 ml) by drip with digoxin (0.13 mg) and epinephrine (2 ml of 1:1,000)
51.2 Penicillin (600,000 u)
67.0 AR off; spontaneously inhaled $O_2$-enriched air
68.0 Removed and cleaned tracheal tube and replaced it
69.5 Dextrose, 5%, ip (225 ml) with epinephrine (1.2 ml of 1:1,000) and digoxin (0.13 mg); penicillin (600,000 u); AR continued
74.1 Tracheal tube removed; AR discontinued and animal placed in $O_2$ tent
74.5 Gasping and head drooping
74.6 Trembling and arms quivering
76.3 Drooling
79.1 Collapsed, lying on side; no response to sound
80.8 Dead (lung congestion)

D. Animal No. 48.

This animal received 5 LD50's of toxin, and the times in hours to effects after toxin administration are as follows:

25.0 Ptosis and weakness
25.3 AT (2,000 u); Kemithal, iv (1 ml)
50.3 Appeared to be in shock; AR (head lower than feet)
65.0 Dead (endotracheal tube plugged)

Animal No. 48 deserves some consideration even though death was caused by an accidental occlusion of the airway 65 hr after the toxin. Regardless of the accident, death would have occurred soon thereafter as a result of pulmonary congestion. The pathology report given below indicates that the lungs were seriously damaged and that death was imminent. This animal did not have any supporting treatment, such as iv and ig feeding, or any of the drugs that the other animals received.

1. External Examination.

Head swollen and edematous (monkey on 30° tilt in resuscitator at all times with head lower than body).

2. Internal Examination.

Fluid collected in tracheobronchial tree; some of this fluid collected in the tracheal tube and dried out, occluded the air passage, and caused death. Lungs were not overinflated and contained some small areas of consolidation.

3. Provisional Anatomical Diagnosis.

Death was caused by suffocation from occluded bronchi.

4. Microscopic Examination.

a. Trachea: The submucosa of the tracheal epithelium is edematous and moderately infiltrated with inflammatory cells consisting of lymphocytes, plasma cells, eosinophils, and PMN's. There are several focal areas where the inflammatory cells penetrate the lamina propria. The submucosal capillaries are congested. The epithelium appears normal over most of its areas, except for one focus extending about one-quarter of the circumference. In this area, the epithelium appears shallow, with loss of cilia. Nuclei along the surface lie longitudinally as in early squamous metaplasia. One small focus exists in this squamous area where the epithelium is denuded down to the lamina propria and is covered in its stead by nuclear debris and PMN's.

b. Lung: The lung sections show alternate areas of emphysema and atelectasis. Around several of the bronchioles, there are large, circular, clear areas that definitely compress the surrounding lung parenchyma and appear to be caused by air under pressure.

5. Final Anatomical Diagnosis.

a. Tracheitis, mechanical.

b. Pulmonary emphysema and atelectasis.

c. Pulmonary congestion.

E. Animal No. 53.

This animal received 5 LD50's of toxin, and the times in hours to effects after toxin administration are as follows:

26.3 Ptosis
30.0 Salivation
30.5 Pentothal, iv (35 mg); AT (2,000 u); Combiotic, im (1 ml); AR; head lower than feet; turned body from side to side after 1 to 2 hr, frequently clearing mouth of excess fluid
46.5 Difficult SR; AVR shut off; $O_2$ given by endotracheal tube
47.0 $O_2$-enriched air in $O_2$ tent
49.4 Nasal feeding, milk mix (50 ml)
50.0 $O_2$-enriched air
52.0 Nasal feeding, milk mix (50 ml)
52.8 Labored breathing, gasping; $O_2$ through endotracheal tube by AVR
53.8 SR; cleaned endotracheal tube and mouth; $O_2$-enriched air through endotracheal tube
54.0 Amino-Plus, iv drip, in saline (30 ml); dextrose, 50%, iv drip (20 ml); Combiotic, im (1.5 ml)
54.5 $O_2$ through tracheal tube by AVR
61.8 SR; placed animal in $O_2$ chamber
63.3 Respiration good
66.0 Respiration difficult; heart sounds normal
70.5 Gasping; can sit up
71.7 Respiration rate, 36/min; Combiotic, im (1.5 ml)
74.5 Labored breathing
75.0 AR by AVR during nasal feeding, milk mix (50 ml); saline, iv drip (195 ml); dextrose, 50%, iv drip (15 ml); Vaponefrin aerosol into trachea (0.3 ml)
76.3 SR irregular, gasping; no eye reflex; $O_2$-enriched air
76.4 AR by AVR
77.3 SR, irregular, gasping-type; moved very little; $O_2$-enriched air
78.0 Dying, no responses
78.3 Dead (lung congestion)

1. Gross Examination.

a. Lungs: Normal, except for small dark areas that covered the surface of portions of both lobes. From a cut lobe, a thick, whitish substance was expressed from the tissues.

b. Liver: Normal.

c. Urinary bladder: Full of urine.

d. Trachea: Contained reddish, mucuslike material. Near the bifurcation, dark-red, stringy mucus clots weighing 15 gm were removed. Some pieces were nearly 1 in. long and plugged all airway passages to the lungs.

F. Animal No. 65.

This animal received 5 LD50's of toxin, and the times in hours to effects after toxin administration are as follows:

30.8 First toxic signs, slight ptosis and muscular weakness
38.8 AT, iv (2,000 u); Surital, iv (2.8 ml); SR
39.0 Placed in respirator; endotracheal tube inserted; aspirated thick, mucuslike material from nose and mouth; repeated aspirations hourly and periodically turned animal from side to side
50.1 Stopped respirator to give nursing care; $O_2$ supplied by AVR via endotracheal tube; dextrose, 5%, ip (200 ml); Combiotic, im (2 ml)
72.0 Powdered-milk mix, ig (100 ml) with Octavitamin (1 ml); dextrose, 5%, ip (300 ml); Combiotic, im (2 ml)
96.0 Powdered-milk mix, ig (100 ml) with Octavitamin (1 ml)
96.5 Dextran, 6%, ip (250 ml); Combiotic, im (2 ml)
97.0 $O_2$ given for 2 min until color improved
122.3 Repeated $O_2$
122.8 Massaged legs; Combiotic, im (2 ml)
123.0 Dextran, 6%, ip (300 ml); $O_2$ by AVR
125.3 Powdered-milk mix, ig (300 ml)
133.3 Became unconscious, failed to respond to $O_2$ therapy; dead. Respirator was working properly and airway was not clogged. Death was attributed to the pulmonary complications of botulism, hypoventilation, atelectasis, and bronchopneumonia.

1. Internal Examination.

a. Body cavities: Several 3- to 4-mm gray-green cystic nodules in the mesentery and omentum.

b. Heart: No significant lesions.

c. Lung: The posterior half of both lungs was blue and airless, with a depressed, wrinkled pleura.

d. Liver: There were numerous 1-mm milky-white nodules in the capsule.

e. Bladder: Rough hemorrhagic serosa.

f. Trachea: Moderate hemorrhagic tracheobronchitis.

2. Microscopic Examination.

a. Lungs: There is extensive collapse with severe congestion and moderately severe bronchopneumonia. The larger bronchi have a tall, actively secreting epithelium and an edematous submucosa with an inflammatory infiltrate.

b. Trachea: There is a severe hemorrhagic and ulcerative tracheitis.

c. Heart: There is a very scanty focal interstitial infiltrate of lymphocytes and some interstitial fibrosis.

d. Liver: There are small collections of normal bile ducts in the hepatic lobules, often just beneath the capsule. There is a small amount of interstitial fibrous tissue, with a few inflammatory cells around these ducts, but hepatic and portal blood vessels are inconstantly nearby. These foci are interpreted as small hamartomatous malformations, similar to the embryonic rests called Myenburg's complexes in man. A few of these are fibrotic. There is also a single regenerative nodule of hepatic parenchyma, with fibrosis of the sinusoids or parasinoidal space.

e. Spleen: There are many plasma cells in the pulp. Since this animal died 6 days after exposure, could this represent antibody formations?

f. Mesentery: There is an encapsulated granuloma containing fragments of dead parasites.

g. Bladder: Subserosal hemorrhage and acute inflammation.

h. Adrenal gland, uterus, and ovary: Normal.

3. Final Anatomical Diagnosis.

a. History of botulism.

b. Congestion and collapse of the lungs.

c. Bronchopneumonia, moderate.

d. Acute hemorrhagic tracheitis.

e. Bile-duct hamartomas of the liver, multiple.

f. Acute cystitis.

g. Parasitic granuloma of the mesentery.

4. Comments.

This animal's death is attributable to the pulmonary complications of botulism, hypoventilation, atelectasis, and bronchopneumonia.

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13. ABSTRACT

Monkeys with respiratory paralysis caused by intravenous administration of 5, 12, and 50 LD50's of type A botulinum toxin (with and without antitoxin) were given artificial respiration (AR) after the appearance of toxic signs. Average times to death were markedly delayed when compared with those of an untreated control group. Animals administered only AR became less responsive, were in a coma during the last day, and eventually died. Spontaneous respiration was restored in two animals that received intermittent AR, antitoxin, and adequate nursing care. Eventual deaths were attributed to lung consolidation or plugging of airway passages. The animals in which spontaneous respiration was not restored died during AR from an oxygen deficiency caused by congestion or consolidation of the lungs. Lives of monkeys with respiratory paralysis caused by lethal doses of type A botulinum toxin can be prolonged by the use of AR (with and without antitoxin), but the method of AR may cause irreversible lung damage during continuous long-term treatment.

14 KEYWORDS

| | | |
|---|---|---|
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